Review of ETP Literature on CHESS

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I. Executive Summary

The Chase Encirclement Stress Studies (CHESS) are aimed at assessing whether the tuna fishery causes sufficient stress to Stenella spp. that might affect their survival and reproduction beyond deaths directly caused by chase and capture. The latter includes directly observed mortality, but set-related unobserved mortality remains unmeasured. The former includes the following stressors: physiological exertion, hyperthermia, social separation, isolation, restraint, injury, and other traumatic events. Chronic stress can adversely affect the immune system and ability to respond to disease, infection and additional challenges (e.g., predation, reproduction, growth, energetic). Recovery of the population from periods of extensive and intensive exploitation (the 1960s-1980s) has been modest at best. The reduction of direct deaths, estimated to be about 0.1% of those encircled, do not account for the poor recovery. The 13 studies reviewed, in sum, suggest a substantial negative impact of purse-seine tuna fishing on Stenella populations. Given that most spotted dolphins are likely to experience on average monthly chases lasting 2-3 hours, such repeated (and thus chronic) acutely stressful events are likely to have a significant impact on survival and reproduction, but estimates of such "impact" remain elusive. Research that directly studied altered immune function and physiological responses (CIE-S02, CIE-S03, CIE-S07, CIE-S08) were most convincing.

II. General Recommendations

- 1. Although tuna fishing operations have markedly improved, operations that set on dolphins clearly qualify as Level A and Level B harassment under the MMPA 1972 given the evidence presented in the CHESS studies.
- 2. Consistent with NMFS 1994 amendments to the MMPA for Fisheries, it is incumbent on the tuna industry to demonstrate that: A) incidental mortality and serious injury due to commercial fishing will have a negligible impact on the affected species or stock; and B) a recovery plan for that species or stock has been developed or is being developed. Currently neither of these necessary conditions for licensing is being met.
- 3. The CHESS studies focus on spotted dolphins that are heavily impacted by tuna fishing. However, as sporadically noted, spinner dolphins, who are also set upon, have more severe reactions and higher mortality in response to the chase and capture. This needs to be assessed.
- 4. Better baseline data from un-impacted populations of *Stenella* are clearly needed for comparison. This includes physiological, demographic, behavioral, and ecological data. Long-term data on *Stenella* have been collected at several sites near the Bahamas. It might be a matter of accessing that information. Although different populations, some similarities are bound to be stable. If at all possible, it might be useful to compare data collected from kills early in the tuna purse-seine operations to later data (However, the techniques may have changed sufficiently to make this less worthwhile.)

- 5. Population models need to include accurate life-history estimates that realistically assess periods of lactation based on studies of wild *Stenella* and other delphinid populations that are under low anthropogenic pressure. For example, several research teams have been studying spotted dolphins in the Bahamas. Perhaps these data can be used for this purpose (e.g., Herzing 1997 finds inter-birth intervals with surviving offspring to be four years, and calves are observed nursing for three years or longer, consistent with other long-term studies of similar sized un-impacted delphinids.) Although they are an ocean away, the life-history characteristics should be similar.
- 6. The fluidity and stability of *Stenella* groups and sub-groups should be determined to assess likelihood of repeat chases and captures of the same animals. There are two ways to do this. First, focal follows of individuals in habituated (but unimpacted animals), such as those studied near the Bahamas or other sites could document patterns of fission and fusion for individuals of varied age or sex class. Second, telemetry techniques could be employed. The social structure may be quite different for the most pelagic populations, but the combined efforts might yield a better picture.
- 7. We need better assessment of how chases, encirclement and captures affect immediate group structure and particularly mother-calf separation. This will provide better measures of "unobserved" mortality.

III. Review of the Literature.

The behavioral ecological perspective was not emphasized in these reports and behavioral sampling was not sufficient to be conclusive. Admittedly, behavioral ecology is extremely difficult to study for pelagic delphinid populations with large and unstable group structure. For comparison, I include a brief paragraph detailing what an "un-impacted" delphinid population is likely to experience with regard to mother-calf separation and acute stress. This is followed by reviews of each manuscript and recommended changes for each.

1. A Behavioral Ecology Perspective

Contrast monthly or even weekly pursuits by helicopters and speed boats followed by encirclement and capture with naturally stressful events, such as predation and conspecific harassment or with naturally occurring mother-calf separations. The population I've been studying for 14 years is similar in body size to Stenella, but is a coastal population with low anthropogenic pressure and considerably different social organization (average group size is 4-5 animals). In 14 years of watching mothers and calves (approximately 2000 focal hours on known mother-calf pairs), mothers and dependent calves are rarely separated (> 10m apart) for more than 25 minutes, even though natural separations of up to several hundred meters can occur 1-2 times per hour (Smolker et al. 1993). Most separations are brief (< 10 min). Separations lasting > 1 hr. occur less than 0.01% of the time, even for dependent calves 3-6 years old (unpublished data). If Stenella were at all similar, then mothercalf separations lasting just half the typical chase, encirclement, backdown and release duration would be highly unusual. This, compounded with acute stress (physiological and social) at a time when calves are likely to seek close maternal contact, suggests that mothers and calves would be hardest hit by tuna fishing operations.

In many thousands of hours of observation of *Tursiops aduncus* in Shark Bay over the last 17 years (including those by other observers), acute stress reactions have

only been observed a few times. Twice it was observed in response to sharks (Connor & Heithaus 1996, Mann & Barnett 1999), once in response to entanglement in fishing line (Mann et al. 1995), and every 3-4 years, cycling females chased and captured by alliances of adult males (see Connor et al. 1996). Even shark encounters (personal observation) and biopsies for genetic sampling (Krützen et al. in press) rarely generate the extreme stress responses described in this report. Further, the duration of such chase and encirclement is far greater than those occurring during predation or biopsy attempts, further exhausting the animals. In sum, it is clear from this report, that other than direct hunting, the intensity, frequency and duration of stressful events associated with purse-seine tuna fishing exceed those for any comparable naturally living delphinid population.¹

2. CIE-S01: Forney et al. "Chase encirclement stress studies on dolphins involved in Eastern Tropical Pacific Ocean Purse Seine Operations During 2001"

This manuscript provides a helpful overview of the CHESS studies and history of the ETP purse seine operations. Strengths and weaknesses of each study are highlighted. There is lengthy discussion of why few lactating females were captured during the CHESS study. Lactation is very difficult to assess (see Oftedal 1997) and likely to be missed if the calf has recently nursed, for example. This has probably contributed to the (likely false) assumption that ETP dolphins only nurse for up to two years. Further, this suggests an even higher proportion of lactating females than found in previous estimates (Archer et al. 2001) and would exacerbate even further the "calf-deficit" found in their research. Thus the unobserved mortality for calves is likely to be greater than Archer et al. report.

Forney et al. conclude that "there is insufficient evidence to conclude that repeated chase and encirclement leads to a state of distress, organ dysfunction, immunosuppression, reproductive failure, or insidious mortality." However, the evidence weighs heavily in this direction. It would be a mistake to assume there is no effect (Type II Error) or only a minor effect. Given the methodological difficulties and small samples for some of the studies, Forney's conclusions should not be misinterpreted to mean that there is *no* evidence, only that more and better data are needed to be conclusive.

3. CIE-SO2: St. Aubin "Hematological and serum chemical constituents in eastern spotted dolphins (*Stenella attenuata*) following chase and encirclement."

As predicted, hormonal changes following chase, encirclement and capture clearly indicate stress, with elevated levels of norepinephrine, dopamine, ACTH and cortisol. St Aubin described a number of methodological limitations in the study, but presented convincing results nevertheless. The long-term effects of these changes are not known, but extensive mammalian literature on chronic stress of this nature, suggests significant impacts on health and reproduction (e.g., Sapolsky et al. 2000). Individual variation in response to stress is not well understood and this needs to be considered.

¹ It could be argued that some delphinid populations are under heavy pressure from tourism or boating activity, e.g., killer whales in Vancouver, spinner dolphins in Hawaii, bottlenose dolphins in Sarasota, Florida. The long-term impacts of human recreational activities are not well understood. However, boating (recreational or tour-operated) are likely to be a chronic, but low-level stressor in comparison to activities associated with purse-seine operations.

4. CIE-S03: Romano et al. "Investigation of the effects of repeated chase and encirclement on the immune system of dolphins (*Stenella attenuata* and *Stenella longirostris*) in the Eastern Tropical Pacific."

Results from this study suggest that T-cells increase and B-cells decrease with repeat chase and capture, and males had higher Class II+ T cells and T helper cells than females. T-cells aid in immune responses and destroy pathogenic cells, while B-cells destroy extracellular pathogens and their products. Since both are indicators of stress, the results tentatively suggest that repeat chase and capture increases spotted and spinner dolphin stress. The direction of effects (i.e., B-cell decrease with repeat capture) are difficult to interpret without knowing more about the fluctuations in immunological function in response to stressors of variable duration and intensity. Regardless, this work makes an important contribution to the small but growing literature on stress in cetaceans.

Given the variation in the sample, it wasn't clear why the "repeat-capture" group wasn't compared with itself using a Wilcoxon Matched-Pairs or a Fisher Matched-Pairs Randomization test. Either would have yielded critical results concerning the effects of capture on the same animals, even though the time window is short. Also, a power test would help indicate if results that were non-significant are primarily due to a small sample size (Type II Error).

Additional information should be added if this manuscript is revised. The Fratios for the ANOVAs are missing. More details on methods should be included, such as how soon blood was taken after capture and why the repeat capture animals weren't compared within sample (from first to later capture). Given the title of the manuscript, it needs to be clear which *Stenella* species are being sampled. Are they all spotted dolphins? The tables and figures need to be labeled more clearly. Besides matching sex, length, and girth, reproductive status would be a critical variable to match the recapture and first-capture samples. With complex interactions between hormonal status and immune system, lactating, pregnant and non-lactating females should be matched in particular. Of course, gathering this information is difficult in the field, but it was gathered in the CHESS studies. Alternatively, if the authors had compared the 10 recaptured animals with each other, this interaction would be controlled for.

Baseline data are obviously needed. "First-capture" is a misleading term when the population has likely experienced repeated captures. In the literature on mammalian stress (e.g., Sapolsky et al. 2000), clearly chronic stress has greater health costs than acute stress. Thus, a one-time capture may not unduly affect the animals compared to repeated captures. It would be naïve to suggest that animals become accustomed to the captures and are thus less affected by them over time. Sex differences in response to stress would also be expected since males and females have markedly distinct hormonal profiles and testosterone, in particular, tends to suppress immune function. However, female reproduction is more likely to be affected by chronic stress than male reproduction. Even without baseline data, the results suggest a significant impact on the *Stenella* populations by repeated chase and capture. It is well established that chronic stress is likely to increase vulnerability to disease and decrease reproductive function.

5. CIE-S04 Pabst et al. "Measuring temperatures and heat flux from dolphins in the Eastern Tropical Pacific: Is thermal stress associated with chase and capture in the ETP-Tuna Purse Seine Fishery?

This study examines the effects of prolonged high-speed chase on dolphin body temperatures. Hyperthermia can adversely affect both male and female reproduction and would thus be an additional cost to dolphins experiencing repeated chase and capture. Pabst and her colleagues have done superb work in this area and are uniquely qualified to examine the issue. They hypothesized that longer chases would result in higher core body temperatures than shorter chases, and that the length of time corralled would cause significantly elevated body temperatures. As Pabst et al. indicate, it isn't just the physiological exertion from the chase, but the fact that dolphins are pursued in surface waters (higher temperature) and when corralled, cannot effectively reduce body temperature.

Contrary to their predictions, deep body temperature was weakly negatively correlated with total chase duration for females but not males. Using 15-minute time blocks, this relationship did not hold, but the "binning" markedly reduced sample size and power of the test. The length of time corralled was not related to deep body temperature for either sex. However, the time help in the processing raft did relate to increasing deep body temperature, but statistical tests weren't applied here.

For the infrared thermography, the main problem seems to be non-independence of samples because individuals were not identified. Thus, multiple photographs of the same animal could contribute to the sample. (The researchers recognized this problem.) However, this problem would affect all the data used in the comparison and relative differences should still hold. The results indicate that longer chases result in higher dorsal fin temperatures and greater differentials between skin and water temperatures. Contrary to their predictions, the time spent in the corral did not correlate with dorsal fin or body temperature or water temperature differentials. However, dorsal fin temperatures did correlate with time spent in the processing raft.

Using a thermal data logger for two animals, the results generally indicate that heat flux values increase following helicopter chase, decline when the chase ends (during capture) and then increase again after backdown and escape. Given equipment and other methodological problems and that only two animals were tagged, these results are inconclusive.

The results generally suggest that dolphins chased for longer periods have increased body temperature and shunt blood to the periphery for cooling. Using a comparable dataset from bottlenose dolphins in Sarasota, Florida, Pabst et al. further conclude that although bottlenose and spotted dolphins have similar core temperatures, dorsal fin temperatures were much higher in corralled ETP spotted dolphins than bottlenose dolphins and even higher for those held in the raft. Contrary to their predictions, time spent in the net did not significantly hamper the spotted dolphins' cooling ability. The long-term effects of chases on body temperature and resulting physiological effects are not known.

6. CIE-S05: Chivers & Scott "Tagging and tracking of *Stenella* spp. during the 2001 Chase Encirclement Stress Studies Cruise."

This study deployed nine VHF radio tags with either time-depth-recorders, time-depth-velocity recorders, or time-depth-velocity-heat flux recorders. The latter were described by Pabst et al. To determine stability of association within groups, Chivers & Scott attached 213 visual tags on spotted dolphins and 8 short-range radio tags (one spinner and seven spotted dolphins). The results generally indicate that

dolphins dive 5-20m during the day and deeper >20m at night. Speeds do vary from day to night but do increase in response to the helicopter and chase.

Specific distances during tracking are not given. Its stated that the boats stayed far enough away to avoid disturbing the animals and that distances shorter than 2 miles from the *McArthur* ship had an effect, but it doesn't state how far the purse seine and *McArthur* boats actually stayed from the animals (although stated in CIE-S01). The stability of group structure is difficult to interpret given the short tracking time for the animals. This work is largely descriptive and its relation to stress induced by the tuna fishery is unclear.

7. CIE-S06: Santurtún & Galindo "Coping behaviors of spotted dolphins during fishing sets."

The aims of this study were to document behavioral changes in relation to group size and to specific set durations (chase, encirclement, capture, backdown), and to examine aspects of set operations during CHESS in comparison to actual tuna fishing operations. Clearly better methods of behavioral assessment need to be developed before such data can be used. The active vs. passive dimension may not be a good indicator of physiological and psychological stress and there are a number of methodological and statistical problems with this study.

Some of the statistical and methodological problems can be corrected. Scan sampling was used to measure group behavior. If this manuscript is revised, the scan method needs to be described in more detail, showing how each individual was selected for the scan. Also, it wasn't clear how the video-taped data were coded. Some of the terms used are not standard for the literature (e.g., rafting is usually used in the same context of floating.) Agonistic interactions are not defined. It's unclear why spy with aerial activity would be classified as a passive event. One-zero sampling is not a valid behavioral measure (see Altmann 1974; Mann 1999), even for comparing prevalence between set variables. In comparing CHESS to tuna fishery captures, the degrees of freedom is 17, but there were only 17 CHESS sets. The sample sizes for tuna fishery captures should be included in Table 2. Its unclear what the sample size was for the Mexican tuna fishery using observer records. Its unclear how the data were combined from the scan samples because sample sizes were not presented in the text or on the figures. Further, its unclear if the figures are showing total percentages of combined data, means, or medians (as they should if Mann-Whitney tests are used, e.g., Figure 1) and there are no error bars (if means are presented). A Bonferroni or other correction is needed given the number of correlations run. The labels in Table 1 make no sense. How could "dolphins in active behaviors during backdown" occur during other set periods? Only 11 sets are presented in Table 3. It's not clear what happened in the other six. The scale the Yaxis of Figure 2 should only go to 100%. Most of these behaviors are not defined in Appendix 1.

There are multiple interpretations of these data, even if we accept the results. For example, moderate swimming in small schools more than large schools may not be a sign of stress; dolphins in large schools may simply be at greater risk of losing track of close associates with faster swimming, and thus be more likely to swim slowly. The authors are aware of these problems. The underwater data are impossible to interpret given no baseline and the one-zero coding of what is essentially ad-lib data.

8. CIE-S07: Dizon et al. "Stress in spotted dolphins (*Stenella attenuata*) associated with purse-seine tuna fishing in the Eastern Tropical Pacific."

Chronic stress from tuna-fishing was measured using molecular changes (stress-responsive protein, SRP) in dolphin skin by comparing 424 samples from fishery carcasses, 202 biopsies of live (bow-riding) animals, and 242 biopsies from dolphins captured during CHESS. The bow-riding animals were assumed to be "uninvolved" in the fishery because animals repeatedly targeted are less likely to approach boats and bow-ride. The other two groups were "involved." The degree of involvement was also assessed using spatial analysis of where U.S. and international sets occurred. The datasets are quite different, but Dizon et al. were careful to control for as many factors as possible. The biggest problem stems from the samples taken from different parts of the body. The fishery samples came primarily from the jaw; the bow-riding samples were primarily dorsal surface and the CHESS samples were from the dorsal fin. Causation is extremely difficult to show, even in the best controlled laboratory studies; yet these results were reasonably convincing (in conjunction with CIE-08) that tuna fishery operations directly or indirectly cause chronic stress in spotted dolphins.

The major difficulties in this dataset include: 1) CHESS patterns did not show a clear relationship to set exposure; 2) fishery data suggest an increase in altered expression with increasing set history but bow-riding data suggest a decrease in altered expression with increasing set history. A power analysis of the CHESS data would indicate if sample size was a problem in the first case. Concerning the second case, other variables may relate to SRP differences between samples. Younger animals may be more likely to bow-ride than what is represented in the "involved" sample. However, since they found no age-related differences in altered expression within the fishery sample, this is of less concern. It may also be true that, as they suggest, healthier dolphins are more likely to bow-ride compared to those less healthy, especially in areas with increasing fishing effort. Finally, as Dizon et al. suggest, sampling from the same body part in future studies is essential.

9. CIE-S08: Southern et al. "Molecular signature of physiological stress based on protein expression profiling of skin."

SRP (Stress response protein) expression profiles were characterized for 871 spotted dolphins exposed to varied levels of tuna fishing pressure (same sample as in CIE-S07) and compared to cetaceans with varied health problems or stress conditions. Spotted dolphins exposed to higher levels of fishing had higher proportions of SRP expression in the skin compared to those with lower fishing exposure. This study added to CIE-S07 with many more details about SRP expression and a comparison group of eight species of wild free-ranging cetaceans. In the bow-riders (low fishery exposure) 37% had altered SRP profiles compared to 83% of the historical fishery samples and 86% of the CHESS samples. Based on where the samples were taken, Southern et al. suggest that the banded SRP pattern represents sporadic stress caused by less fishery pressure compared to the uniform pattern, possibly indicative of chronic and prolonged stress. Further investigation of this marker would be useful. In addition, there was little discussion of the "Relative Expression Level Scale" in the text. The data were analyzed on a one-zero basis (normal, stressed) and the continuous scale might be useful and how it interacts with the banded pattern.

Southern et al. offer a possible explanation of the counter-intuitive result of fewer altered SRP profiles after repeated capture. They suggest that in the short-term, SRP patterns may respond to corticosterone surges and heat shock proteins. This

needs more study. Further, that SRPs can also be profiled from mucosal tissues, semen, milk and blood offer additional avenues for sampling, but these would all require capture. Finally, the relation between SRP profile and body part sampled (i.e. dorsal fin, dorsum, jaw) needs to be resolved.

10. CIE-S10: Romano et al. "Investigation of the morphology and autonomic innervation of the lymphoid organs in the pantropical spotted, spinner, and common dolphins..."

Lymphoid organs (spleen, mesenteric lymph node, thymus, gut-associated lymphoid tissue and lymph nodes) collected from 57 incidental dolphins caught in the tuna fishery (1999-2001) showed no signs of tissue involution, normally indicative of stress. Although age estimates are given, teeth were not analyzed, so the ages must be interpreted with caution, especially for spinner and common dolphins that do not show marked pigmentation changes with age. Not all lymphoid organs were examined on a consistent basis, thus sample sizes are small for specific organs (e.g., two thymus samples). It is unclear whether some lymphoid organs might show greater stress responses than others. And, as the authors point out, the effects of stress are likely to be highly time dependent. The authors make a number of good suggestions for refining this work. At present, the results are inconclusive.

11. CIE-S11: Cowan & Curry "Histopathological assessment of dolphins necropsied onboard vessels in the Eastern Tropical Pacific Tuna Fishery."

Tissue samples of 56 dolphins (spotted, spinner and common) caught during tuna purse-seine fishing (1999-2001) were examined for cause of death and stressrelated histopathology. Most animals died from acute shock (cardiac arrest). In itself, this would suggest that chase and capture could be stressful enough to cause death. They suggest that cetaceans may be particularly vulnerable to acute endogenously generated myocardial injury because of their diving physiology. In sum, perceived threat triggers an alarm (escape) response when the physiological (flight) reaction is not possible. For a diving mammal, the flight response involves breath-holding and re-directing blood from non-vital to vital oxygen-dependent organs such as the brain and heart. Under threat, a massive release of adrenergic hormones (and release of catecholamines) can cause spasm of coronary arteries, followed by myocardial ischemia, arrhythmia and death. Thirty-six percent of the animals in the current sample showed histopathological evidence (myocardial scars and abnormalities) of prior stress related to chase and encirclement operations. The immune systems were otherwise functioning adequately and parasite levels were considered lower than coastal populations (which tend to have greater parasite exposure). The article provided an excellent review of cetacean stress literature.

12. CIE-S12: Edwards "Potential effects of chase and encirclement by purseseiners on behavior and energetics of spotted dolphin (*Stenella attenuata*) mother-calf pairs in the ETP"

Given the energetic demands on lactating females (increase in 50% food intake during lactation) and the limited energy reserves of their calves (especially neonates), mother-calf pairs are probably most vulnerable to harassment from purseseine tuna fisheries. In addition, this class of individuals is most critical to population recovery. Edwards reviews early development in dolphins and factors that might increase maternal and calf vulnerability to fishing activities. Although the overall picture is correct, some of the cited literature needs to be reviewed more carefully.

Calves have reduced levels of myoglobin, lesser diving capability, and less stamina compared to adults. Neonates may not "recognize" their mothers for the first week (*Tursiops*, Mann and Smuts 1998) and would be most likely to become permanently separated during the confusion of fishing operations, especially those involving large groups of dolphins (which are set on as often as every week). In addition, with chases that typically last for thirty minutes and sometimes for over an hour, calves would be unable to "keep up" and would inevitably lag behind the group. Risk of shark predation is likely to exponentially increase given reports of sharks attracted to purse-seine operations, and the prevalence of separated and exhausted calves. Calves are most likely to be represented in set-related "unobserved" mortality (Archer et al. 2000). Orphaned calves are unlikely to be adopted and are unlikely to survive independently unless they are at least three years of age. Heat stresses to pregnant females are likely to adversely affect the fetus (see CIE-S04).

13. CIE-S13: Edwards "Energetics consequences by tuna purse-seiners for spotted dolphins (*Stenella attenuata*) in the eastern tropical Pacific Ocean"

Using standard hydrodynamic and energetic models, this study estimates the energy expenditure of mothers and calves during chases by tuna fisheries. Methods using free-swimming *Stenella* were not considered feasible, so the models were based on *Tursiops* and other mammals, and basic energetic equations (e.g., hydrodynamic drag, thrust, power). Edwards estimates that for a 15-minute *slow* chase (3 m/sec), dolphins would increase caloric expenditure by 3%, by 6% for a 30-minute chase and by 12% for a 60-minute chase. Chase duration and higher chase speeds become costly more quickly, especially for smaller dolphins. At increased speeds of 7.5m/sec, an adult would increase caloric expenditure by 90% for a 30 min. chase, and a neonate would expend 160% more. In addition, the mother with a drafting calf would experience greater energetic expenditure ranging from 16%-48% increase, depending on the size of the calf. Calves are unable to sustain themselves during high-speed chases and likely fall behind the group.

Its assumed in the manuscript that calves older than 3 mos. would not draft during chases. This needs to be assessed. Calves may continue to rely on their mothers' draft during stressful events up until weaning (at 3 yrs., based on Herzing's *Stenella* population, Herzing 1997 and our *Tursiops* population with similar sized dolphins Mann *et al.* 2000). Calves older than three months typically swim in "infant position" under the mother, lightly touching her abdomen, but move to the drafting position when moving fast (e.g., towards a large fish school). Thus, it cannot be assumed that captive situations are representative, since captive animals, by definition, cannot really travel anywhere. However, it may be that drafting relationships cannot be sustained during prolonged high-speed swims for reasons outlined by Edwards.

Given convincing evidence that mothers and calves are likely to become separated during chase and capture events, what is the likelihood of reuniting? This may depend on a host of factors. The larger the school, the more confusing and difficult this would be. The duration of the chase would affect the distance of separation. Interfering noise of boat engines and other whistling dolphins would mask the ability to hear one's own mother or offspring (assuming that the whistles are individually recognizable, something that has not been assessed in this population).

Although the recommendations would reduce mortality risk for mothers and calves, the first, to avoid setting on schools that contain calves, is probably not feasible. Nearly all spotted dolphin groups are likely to contain calves.

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APPENDIX 1: MATERIAL PROVIDED BY THE SOUTHWEST FISHERY SCIENCE CENTER

CIE-SO1. Chase encirclement stress studies on dolphins involved in Eastern Tropical Pacific Ocean Purse Seine Operations during 2001. KA Forney, DJ St Aubin, SJ Chivers.

CIE-S02. Hematological and serum chemical constituents in Eastern Spotted Dolphins (*S. attenuata*) following chase and encirclement. D St Aubin.

CIE-S03. Investigation of the effects of repeated chase and Encirclement on the immune system of Dolphins (*S. attenuata and longirostris*) in the Eastern Tropical Pacific.

T Romano, M Keogh, K Danil

CIE-SO4. Measuring temperatures and heat flux from dolphins in the Eastern Tropical Pacific: is thermal stress associated with chase and capture in the ETP-Tuna Purse Seine Fishery ?D. Ann Pabst, WA McLellan, EM Meagher, AJ Westgate.

CIE-SO5. Tagging and tracking of *Stenella* spp. during the 2001 Chase Encirclement stress studies cruise. SJ Chivers, MD Scott

CIE-SO6. Coping behaviors of spotted dolphins during fishing sets. E Santurtún and F Galindo

CIE-SO7. Stress in spotted dolphins (*Stenella attenuata*) associated with Purse-seine Tuna fishing in the Eastern Tropical Pacific. A Dizon, A Allen, N Kellar, S.Southern.

CIE-SO8. Molecular signature of physiological stress based on protein expression profiling of skin. Š. Southern, A Allen, N Kellar, A Dizon.

CIE-S10. Investigation of the morphology and autonomic innervation of the lymphoid organs in the Pantropical Spotted, Spinner, and Common Dolphins incidentally entangled and drowned in the tuna purse-seine fishery in the eastern tropical Pacific. T Romano, K Abella, DF Cowan, B Curry.

CIE-S11. Histopathological assessment of dolphins necropsied onboard vessels in the Eastern Tropical Pacific Tuna fishery. DF Cowan, BE Curry

CIE-S12. Potential effects of chase and encirclement by purse-seiners on behavior and energetics of spotted dolphin (*S. attenuata*) mother-calf pairs in the ETP. EF Edwards

CIE-S13. Energetics consequences of chase by tuna purse-seiners for spotted dolphins (*S. attenuata*) in the Eastern Tropical Pacific Ocean. EF Edwards

CIE-S14. Evasive behavior of Eastern tropical Pacific Dolphins relative to effort by the tuna purse seine fishery. SL Mesnick, FI Archer, AC Allen, AE Dizon.

CIE-S15. Estimation of reproductive and demographic parameters of the eastern spinner dolphin (*S. longirostris orientalis*) using aerial photogrammetry. K Cramer, WL Perryman.

APPENDIX 2: STATEMENT OF WORK

STATEMENT OF WORK

Consulting Agreement Between The University of Miami and Dr. Janet Mann

Background

The tuna industry has used the association between tuna and dolphins to fish in the eastern tropical Pacific Ocean for over five decades. Three stocks of dolphins were depleted by high historical levels of dolphin mortality in tuna purse-seine nets, with an estimated 4.9 million dolphins killed during the fourteen-year period 1959-1972. After passage of the Marine Mammal Protection Act in 1972 and the increased use of equipment designed to prevent dolphin deaths, mortality decreased gradually during the late 1970s, 1980s and 1990s. While changes in the fishery have greatly reduced the observed mortality of dolphins, there continues to be concern that the fishing methods used are causing stress to the dolphins involved and that such stress may be having a significant adverse impact on population recovery. As a result, the International Dolphin Conservation Program Act (IDCPA) required that research consisting of population abundance surveys and stress studies be conducted by the National Marine Fisheries Service to determine whether the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock". The stress studies mandated in the IDCPA include:

- A. A review of relevant stress-related research and a 3-year series of necropsy samples from dolphins obtained by commercial vessels.
- B. A one-year review of relevant historical demographic and biological data related to the dolphins and dolphin stocks.
- C. An experiment involving the repeated chasing and capturing of dolphins by means of intentional encirclement.

The necropsy program (A) has analyzed samples from about 50 dolphins killed incidentally during fishing operations. Historical biological samples and data (B) have been analyzed at the Southwest Fisheries Science Center (SWFSC) to investigate stress-activated-proteins (SAPs) in the skin in dolphins killed in the fishery and live-sampled via biopsy. Historical data were also examined to assess separation of cows and calves during fishing operations. The Chase Encirclement Stress Studies (C; CHESS) were conducted during a 2-month research cruise aboard the NOAA ship McArthur in the eastern tropical Pacific Ocean from August -October 2001. During this project, the team worked in cooperation with a chartered tuna purse seiner to study potential effects of chase and encirclement on dolphins involved in tuna purse seine operations. Dolphins groups were found to be much more dynamic than previously recognized, making it extremely difficult to recapture groups of dolphins over the course of several days to weeks, as planned. In the end, nine different dolphins were tracked for 1-5 days during the course of the study, including two animals outfitted with a thermal tag that recorded heat flux, temperature, and dive data. Individual radio-tagged dolphins and 1-4 associated rototagged dolphins were recaptured on several occasions spanning shorter periods of 1-3 days. Six satellite tags were deployed to record movement and dive data on dolphins that were not recaptured.

Biological data and samples were collected from as many captured dolphins as possible, and include: 70 blood samples, of which 18 were from repeat captures of marked individuals; 283 skin samples, of which 17 were from previously captured and sampled animals; 449 analyzable thermal images; 52 core temperatures; and 95hrs of heat flux data. Females with calves were noted on several recapture occasions, and one known calf was skin sampled during an initial and subsequent capture. All samples and data are being analyzed at SWFSC and other contracted laboratories.

General Topics for Review

This review includes a suite of studies subsumed under the general topic of "Stress Studies". Up to 17 separate papers will be provided covering the studies described below. The general components are as follows:

- Necropsy samples: Analysis of tissues from dolphins incidentally killed in the fishery.
- Blood samples: Analysis of blood samples collected from wild dolphins captured using purse seine methods to assess A) general health, B) immune function, and C) stress response to capture.
- Stress-activated protein studies: Analysis of skin samples to assess levels of stress-activated proteins in dolphins that were A) killed in the fishery B) captured once C) captured repeatedly and D) bow-riding research vessels.
- Thermal studies: Analysis of thermal images, deep core temperatures, and heat flux data derived from thermal tag deployments on wild dolphins.
- Fishery-related behavior: Analysis of behavioral data from dolphins captured using purse seine methods.
- Behavioral ecology: Analysis of tracking data for dolphins captured, tagged, tracked and recaptured during field studies, to investigate school dynamics and movement patterns.
- Cow/calf separation: Analysis of composition of dolphin schools to investigate separation of lactating females and their calves.
- Dolphin swimming energetics: Analysis of the energetic costs of being chased, particularly for lactating females and associated calves.

Documents supplied to reviewers will include draft manuscripts on topics listed above, and a number of background papers (relevant publications and reports).

Specific Reviewer Responsibilities

The reviewer's duties shall not exceed a maximum total of seven days, including several days to read all relevant documents, and several days to produce a written report of the reviewer's comments and recommendations. It is expected that this report shall reflect the reviewer's area of expertise; therefore, no consensus opinion (or report) will be required. Specific tasks and timings are itemized below:

- 1. Read and become familiar with the relevant documents provided in advance;
- 2. No later than April 1, 2002, submit a written report of findings, analysis, and conclusions. The report should be addressed to the "UM Independent System for Peer Reviews," and sent to David Die, UM/RSMAS, 4600 Rickenbacker Causeway, Miami, FL 33149 (or via email to ddie@rsmas.miami.edu).

Signed	Date
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